EAST AFRICAN COMMUNITY

EDICT OF GOVERNMENT

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EAS 217-7 (2001) (English): Methods for microbiological examination of foods — Part 7: Method of examination for clostridium botulinum and clostridium botulinum toxin



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EAST AFRICAN STANDARD

Methods for microbiological examination of foods — Part 7: Method of examination for clostridium botulinum and clostridium botulinum toxin

EAST AFRICAN COMMUNITY

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Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to achieve this objective, the Partner States in the Community through their National Bureaux of Standards, have established an East African Standards Committee.

The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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Methods for microbiological examination of foods — Part 7: Method of examination for clostridium botulinum and clostridium botulinum toxin

1 Scope

This standard method describes the reference procedure for the detection and confirmation of *Clostridium botulinum* and its toxins in food and culture supernatants.

2 Outline of the method

During growth, the various strains of *C.botulinum* produce toxins which are lethal to man, mouse, and other animals.

The organism and the toxin can be detected and identified by protecting test animals (i.e. mice) against the test material with the specific antitoxin.

NOTE 1 *Special Safety Precautions* — Botulinum toxin is extremely toxic and special care shall be taken when handling foods or culture preparations suspected of containing it. The toxin is absorbed rapidly through the eyes and nose.

NOTE 2 At the conclusion of the test, ensure that all materials used in the test, such as media, reagents, unwanted test samples, apparatus and animals, are rendered safety by incineration or autoclaving.

NOTE 3 It is also advisable that workers handling such materials be immunized with polyvalent botulinum toxoid as a prophylactic before working with these materials.

3 Media and reagents

The following media and reagents are required:

i) Cooked meat medium (CMM)

ii) Differential reinforced clostridial medium (DRCM), Gelatine phosphate buffer (pH 6.5)

iii) Trypsin, 20 g dissolved in 1 litre of gelatine

iv) phosphate buffer (pH 6.5)

v) White mice of approximately 20 g each

4 Testing of suspected samples

a) Macerate the sample (25 g if available) using sterile mortar, pestle and sand.

NOTE Any remaining sample shall be stored below -15°C for further testing if required

b) Add an equal amount of sterile gelatine phosphate buffer (pH 6.5) and mix. Centrifuge to maintain a centripetal acceleration of 35000 m/s^2 (3500 X force of gravity) for 30 min preferably in a refrigerated centrifuge.

c) Collect the supernatant liquid and use undiluted to detect toxin *(see* **6**) .

NOTE After use, the remainder shall be stored below -15 ºC: for further testing if required

d) Collect the sediment and use for the isolation of *C. botulinum* by enrichment culture.

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5 Culturing of *C. botulinum*

5.1 In the isolation of *C. botulinum* from samples likely to contain spores of that organism, it is an advantage to heat the samples to destroy miscellaneous contaminants.

However, spores of types *E* and non-proteolytic type *F* are relatively heat labile; further, the suspected sample may not contain *C. botulinum* spores at all. For these reasons, the samples to be cultured shall comprise:

a) unheated samples;

b) samples heated to 60°C; and

c) samples heated to 80°C.

When adequate sample material is available, treat as follows; if not, reduce the number of tubes used in the procedure:

a) Freshly steam or boil nine tubes of CMM or DRCM in boiling water to drive off oxygen; cool rapidly in cold water.

b) Place three tubes in a 90 ± 1°C water bath and three in an 80 ± 1°C water bath and allow to equilibrate to bath temperature. Leave three tubes unheated.

c) Inoculate about 1 g or 1 ml of either the suspected sample or the sediment collected (see Clause 4 d)) into each of the nine tubes below the surface of the cooked meat layer. Maintain the three tubes preheated to 60°C for 15 min at 60°C and those preheated to 80°C for 10 min at 80°C in their respective water baths. After heating, cool in iced water.

d) Incubate at 30 ± 1°C for 5 days. If a negative report is obtained at 5 days, extend the incubation to a total of 21 days.

 Toxin is usually detectable within 48 h to 96 h and as a rule is in the highest concentration after the period of active growth and gas production.

e) Centrifuge approximately 5 ml of the culture obtained in the preceding step, maintaining a centripetal acceleration of 35000 m/s^2 (3500 X force of gravity) for 15 min; use the supernatant for the detection of botulinum toxin, according to the procedure described in Clause 6.

6 Detection of toxin

The generally accepted procedure for detecting the presence of botulinum toxin is the mouse test:

a) Prepare the following dilutions of food macerate supernatant (see Clause 4 (a)) using gelatine phosphate buffer (pH 6.5): undiluted, 1 in 5, 1 in 10, 1 in 100

 The same dilutions are used for testing culture supernatants (see Clause 5 (e)).

b) Prepare trypsin-treated test dilutions by mixing equal volumes of the sterile 2 per cent trypsin solution with undiluted supernatant and with the same dilutions as those prepared according to Clause 6 a); incubate at 37 ± 1°C for 1 h.

c) From each untreated dilution (including the undiluted material) prepared as in a), inject 0.2 ml intraperitoneally into at least two mice. From each trypsin-treated dilution, prepared as in b), inject 0.4 ml intraperitoneally into at least two mice.

d) Heat undiluted macerate supernatant or culture supernatant for 5 min at 100 °C and inject 0.2 ml intraperitoneally into two mice as controls.

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e) Record the condition of the mice at regular intervals over a period of five days. Provided the controls mice survice, death of the others with typical symptoms is indicative of.botulinum toxins.

NOTE 1 Typical symptoms are progressive inactivity, flaccid paralysis (particularly of the hind legs) , increasing respiratory difficulty and constriction of the abdomen.

NOTE 2 When non-typical symptoms or 'non-specific' deaths occur the initial tests shall be repeated with the addition of 10 per cent mixed clostridial anti-serum and either 220 ppm of chlorotetracycline or 1000 ppm streptomycin sulphate.

NOTE 3 If trypsin treatment has increased the titre or the presence of type *E* or non-proteolytic types *B* and *F* strains is to be suspected. If not, type *A* and proteolytic type *B* are likely to be present.

f) Record the presence or absence of toxin in the test material

7 Typing of toxins

a) Dilute an aliquot of culture supernatant or food macerate supernatant of known toxicity, as determined according to Clause 6, with gelatine phosphate -buffer (pH 6.5) so that the titre of the toxin is equal to or slightly less than the titre of the antitoxin used to protect the mice.

b) For each toxin type, protect two mice by injecting 0.5 ml of the appropriate monovalent type antitoxin into each.

c) Two unprotected mice are used as controls.

d) Approximately 1 h after injection of antitoxic, inject 0.5 ml of the previously prepared toxic test dilution (see a) above) into each of the test and control mice.

e) Record the condition of the mice, as described in Clause 6 e).

If the protected mice live, the type of antitoxin which gives protection corresponds to the type of toxin present.

Samples of meat and vegetables are likely to contain types *A* and *B* spores, and samples of marine products type *E.*

NOTE Most human cases of botulism are caused by ingestion of types *A, B, E,* or *F* toxins. Botulism in animals is usually caused by types *C* and *D.* Therefore, when investigating a human case of botulism, it is advisable to test for at least types *A, B, E* and *F* toxins. If antisera to types *C* and *D* are available, these may be included.

8 Report

Report the absence or presence of *Clostridium botulinum,* its toxigenic type and its toxins.

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